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## Research paper

Linking the concentrations of itraconazole and 2-hydroxypropyl- $\beta$ -cyclodextrin in human intestinal fluids after oral intake of Sporanox<sup>®</sup>Philippe Berben<sup>a</sup>, Jef Stappaerts<sup>a</sup>, Matthias J.A. Vink<sup>b</sup>, Elena Domínguez-Vega<sup>b</sup>, Govert W. Somsen<sup>b</sup>, Joachim Brouwers<sup>a</sup>, Patrick Augustijns<sup>a,\*</sup><sup>a</sup> Drug Delivery and Disposition, KU Leuven, Gasthuisberg O&N II, Herestraat 49 – box 921, 3000 Leuven, Belgium<sup>b</sup> Division of BioAnalytical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

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## ABSTRACT

In a previously performed small-scale clinical study, healthy volunteers were asked to ingest an oral solution of itraconazole (Sporanox<sup>®</sup>) containing 40% 2-hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) (i) with or (ii) without a standardized volume of water (240 mL) after which gastrointestinal and blood samples were collected. Although omitting water during the administration of Sporanox<sup>®</sup> resulted in noticeably higher duodenal concentrations of itraconazole, systemic exposure was almost unaffected. It is assumed that this discrepancy can be explained by differences in the extent of entrapment of itraconazole in the duodenum caused by differential complexation depending on the concentration of cyclodextrins. To further substantiate this hypothesis, the quantification of HP- $\beta$ -CD concentrations in the aspirated intestinal fluids was performed by LC-MS/MS. When comparing the intestinal concentrations of itraconazole and HP- $\beta$ -CD for one single healthy volunteer (HV02) in both test conditions, an excellent correlation was observed (Spearman's rank coefficient of 0.96). Moreover, the data suggest that, similar to aqueous buffer media, also in human intestinal fluids a non-linear relationship exists between itraconazole solubility and HP- $\beta$ -CD concentration ( $A_p$ -type profile; Spearman's rank coefficient of 0.78), indicating that higher order complexes are formed at higher concentrations of HP- $\beta$ -CD. This difference in extent of entrapment in the inclusion complexes helps to understand the observed impact of water intake on precipitation and permeation behavior of itraconazole in man. Without water intake, higher HP- $\beta$ -CD concentrations resulted in less precipitation and increased duodenal concentrations of itraconazole. On the other hand, the stronger interaction at higher HP- $\beta$ -CD concentrations reduced the free fraction of the drug explaining that increased intraluminal concentrations of itraconazole were not translated into an enhanced uptake. In conclusion, quantifying the concentrations of the solubilizing agent HP- $\beta$ -CD in human intestinal fluids appeared to be of crucial importance to interpret the intraluminal behavior of an orally administered cyclodextrin-based solution.

## 1. Introduction

One of the most important contemporary challenges in drug development is the increasing proportion of active pharmaceutical ingredients (APIs) exhibiting poor biopharmaceutical properties [1,2]. For drugs intended for oral administration, enabling formulations are commonly used to meet the prerequisites of attaining suitable drug concentrations and sufficient intestinal transport at the site of absorption. While the use of excipients which affect intestinal permeability remains controversial and usually evokes safety concerns [3,4], a considerable armamentarium of established solubility enhancing techniques is available to the formulation scientist [5].

To enable the non-clinical evaluation of these formulations in a

reliable manner, major efforts are ongoing to design *in vitro* and *in silico* tools that are predictive for *in vivo* gastrointestinal drug behavior [6–8]. Notwithstanding the indisputable progress in this field, it is generally recognized that the correlation between *in vitro* or *in silico* work and the *in vivo* situation is sometimes flawed [9,10]. Especially for *in vitro* models, it is difficult to strike the right balance between an experimental design that is straightforward enough to be implemented for screening purposes and a set-up that oversimplifies the intricate *in vivo* environment. In this regard, techniques that allow gaining insight into the gastrointestinal variables affecting drug and formulation behavior *in vivo* are beneficial as they allow optimizing and validating *in vitro* techniques and offer valuable data to implement in computational absorption models [11]. Among the methodologies available to study

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gastrointestinal drug disposition *in vivo*, intraluminal sampling has been successfully applied to acquire information on composition of gastrointestinal fluids, gastrointestinal drug behavior and impact of gastrointestinal motility [12–14]. Monitoring gastric and/or intestinal drug concentrations upon administration of drug products to healthy volunteers allows evaluating the *in vivo* performance of formulations generating important feedback for *in vitro* and *in silico* tool development. For instance, the administration of Zytiga® (abiraterone formulated as the ester prodrug abiraterone acetate) to healthy volunteers underscored the importance of providing dissolution media with hydrolytic capacity for prodrug evaluation [15]. Indeed, fast intestinal dissolution and subsequent degradation of abiraterone acetate by hydrolytic enzymes triggers abiraterone supersaturation. These mechanisms were shown to be important for the performance of the formulation.

It is obvious from the previous example that a delicate interplay between the formulation and the gastrointestinal physiology takes place after oral intake of a drug. Therefore, it is sometimes difficult to discriminate between formulation effects and physiological processes. A possible approach to establish a clear, unequivocal relationship between the drug concentrations measured *in vivo* and the formulation is the assessment of essential excipients that may affect drug concentrations. For instance, Brouwers et al. demonstrated the importance of measuring the concentration of the solubilizing agent d- $\alpha$ -tocopheryl polyethyleneglycol 1000 succinate (TPGS) in duodenal fluids aspirated from healthy volunteers upon administration of a standard formulation of amprenavir (Agenerase®) to explain the high intestinal concentrations of amprenavir [16].

Recently, we investigated the effect of dilution (administration with or without a glass of water) on the formulation performance of Sporanox® oral solution which contains the antifungal agent itraconazole solubilized by 2-hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) [17]. Itraconazole is a poorly soluble, basic compound and its solubility was demonstrated to improve strongly in the presence of HP- $\beta$ -CD [18]. In addition to this formulation-based effect, the solubility of itraconazole is also significantly influenced by pH. Indeed, *in vitro* and *in vivo* studies clearly demonstrate a superior solubility in the pH range that is relevant for the fasted stomach as compared to the more neutral pH range of the small intestine resulting in itraconazole supersaturation upon gastrointestinal transfer [17,19–21]. In our recent work, we showed that intraluminal dilution of the cyclodextrin-based itraconazole formulation with coadministered water does not affect the systemic exposure to itraconazole, even though duodenal concentrations are reduced. It was argued that this is most likely due to stronger entrapment of itraconazole in cyclodextrin complexes upon administration of Sporanox® without water. Thorough understanding of these observations requires insight into the concentrations of HP- $\beta$ -CD in the intraluminal fluids. The aims of the current study were therefore (i) to quantify HP- $\beta$ -CD concentrations in the intestinal fluids aspirated upon intake of Sporanox® by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and (ii) to explore the link between the intestinal concentrations of itraconazole and HP- $\beta$ -CD.

## 2. Materials and methods

### 2.1. Chemicals

Itraconazole and HP- $\beta$ -CD (Kleptose® HPB parenteral grade, Roquette Frères, Lestrem, France) were kindly provided by Johnson & Johnson Pharmaceutical Research and Development (Beerse, Belgium). Dimethyl sulfoxide (DMSO) and methanol (MeOH) were obtained from Acros Organics (Geel, Belgium). Sodium acetate trihydrate and acetic acid were purchased from VWR (Leuven, Belgium), while acetonitrile (ACN) was supplied by Fisher Scientific (Leicestershire, UK). Formic acid ( $\geq 99\%$ ) and methanol absolute ( $\geq 99.98\%$ ) were purchased from Biosolve (Valkenswaard, The Netherlands). Ammonium formate

( $\geq 99\%$ ) was supplied by Sigma-Aldrich (Steinheim, Germany). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

### 2.2. Clinical trial – study design

The duodenal samples in which the concentrations of HP- $\beta$ -CD were determined, were obtained from a clinical study recently described by Berben et al. [17]. Despite this cross-over study was performed in 5 healthy human volunteers (HV) (3 males and 2 females aged between 22 and 27 years old), only duodenal samples from one single volunteer (HV02) were used to measure the concentrations of HP- $\beta$ -CD. Volunteers could only participate in the study, performed at the University Hospitals Leuven, after a medical examination. Participation of volunteers suffering from gastrointestinal disorders or infectious diseases (hepatitis B or C and HIV) was strictly forbidden, protecting the well-being of the researchers and volunteers. Other exclusion criteria were the regular intake of medication, frequent exposure to X-rays or a potential pregnancy. The clinical study protocol (S53793) was in accordance with the declaration of Helsinki and approved by the Committee of Medical Ethics of the University Hospitals Leuven (ML7919, EudraCT reference number 2011-005928-17) and by the Federal Agency for Medicines and Health Products (FAHMP). All volunteers provided written informed consent before the start of the clinical trial. After an overnight fast (no food consumption and only drinking of water for 12 h), two double lumen catheters (Salem Sump™ PVC Gastroduodenal Tube, 14Ch (4.7 mm) 108 cm, Covidien, Dublin, Ireland) were introduced via the nose and positioned in the antrum (in front of the pylorus) and in the duodenum (D2/D3), guided by fluoroscopy. After an equilibration period of 20 min, 20 mL of Sporanox® solution (10 mg/mL; 200 mg of itraconazole) was orally administered with or without a glass of water (240 mL). Subsequently, gastrointestinal fluids were aspirated (< 4 mL sampling volume) at predetermined time points (7, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 min) for the determination of dissolved and total concentrations of itraconazole and the dissolved concentrations of HP- $\beta$ -CD. Directly after aspiration of the gastrointestinal fluids, pH was measured (Hamilton Knick Portamess®, Bonaduz, Switzerland). For each individual intestinal aspirate, thermodynamic solubility of itraconazole was determined according to the shake-flask method. We would like to refer to the first manuscript for the specifications of the analysis of itraconazole [17].

For the assessment of HP- $\beta$ -CD, aspirated intestinal samples from HV02 were transferred to Vrije Universiteit Amsterdam (VUA) (cfr. Section 2.4.) according to an agreement for inter-institutional transfer of human tissue samples.

### 2.3. Caco-2 permeation of itraconazole from human intestinal fluids

To evaluate the permeation potential of itraconazole from aspirated intestinal fluids, transport of itraconazole from selected samples was determined across Caco-2 cell monolayers immediately after aspiration. To this end, 1.5 mL of TPGS (0.2% w/v) was applied to the basolateral compartment and 100  $\mu$ L of mucus (50 mg/mL) was added to the apical compartment, followed by the addition of 500  $\mu$ L of the aspirated intestinal fluid. Subsequently, samples (100  $\mu$ L) were time-dependently withdrawn during 30 min from the basolateral compartment. TEER values were measured before and after the transport experiment to demonstrate monolayer integrity. For a detailed description of the experimental conditions, we refer to Berben et al. [17].

### 2.4. Analysis of HP- $\beta$ -CD in human intestinal fluids of the fasted state

To quantify the concentrations of HP- $\beta$ -CD in the human intestinal fluids, an LC-MS/MS method was developed. The LC-MS system consisted of a Thermo Finnigan LC system (Thermo Finnigan, San Jose, CA, USA) coupled to an ion-trap XCT 6330 mass spectrometer (Agilent

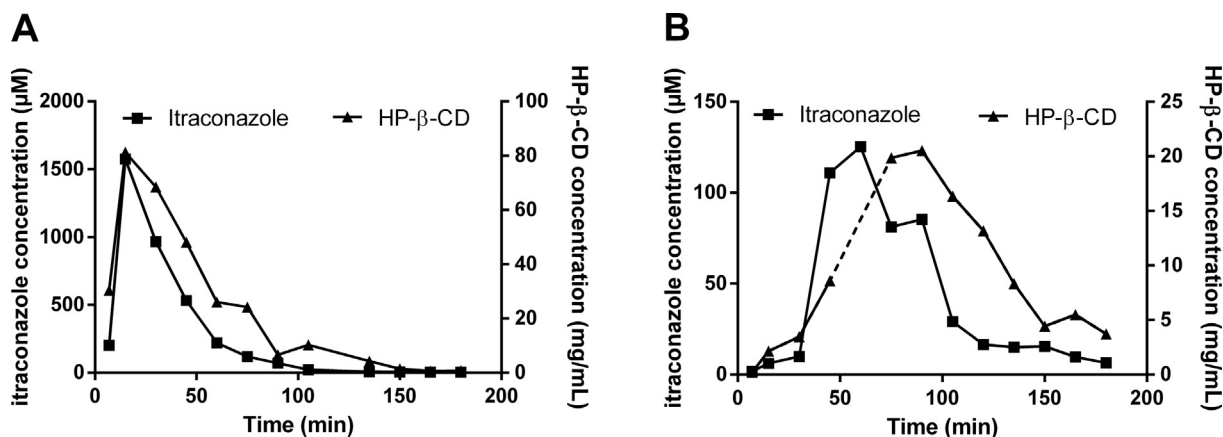


Fig. 1. Duodenal concentration-time profiles of itraconazole (squares, left y-axis) and HP- $\beta$ -CD (triangles, right y-axis) following the oral intake of Sporano<sup>x</sup> without (A) or with water (B) to HV02. The concentration of HP- $\beta$ -CD could not be measured at  $t = 60$  min which is illustrated by a dotted line (B). Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).

Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) source. Cyclodextrin separation was performed using a Waters Xbridge BEH C18 ( $3.0 \times 100$  mm;  $2.5 \mu\text{m}$ ; Waters, Milford, MA, USA). The mobile phase was a mixture of 5 mM ammonium formate in water (solvent A) and methanol (solvent B). The following gradient elution program was applied at a constant flow rate of  $200 \mu\text{L}/\text{min}$ : 1 min at 5% B followed by a linear change to 70% B in 1 min, 6 min of isocratic elution at 70% B, a linear change to 95% B in 1 min, and isocratic elution at 95% B for 6 min, after which the column was re-equilibrated with the initial conditions (5% B) for 7 min prior to the next injection. The sample injection volume was  $10 \mu\text{L}$ . Separation temperature was set at  $30^\circ\text{C}$ .

MS analysis was performed in positive ionization mode using the following settings: ESI voltage,  $3.75 \text{ kV}$ ; dry gas temperature,  $320^\circ\text{C}$ ; dry gas flow rate,  $6.5 \text{ L}/\text{min}$ ; nebulizer pressure,  $12.5 \text{ psi}$ ; skimmer,  $40 \text{ V}$ . A maximum accumulation time of  $50 \text{ ms}$  was set with a Smart ICC of 30,000 to limit the number of accumulated ions dynamically. For MS/MS experiments, the  $1442.3 \text{ m/z}$  ion (ammonium adduct of  $(\text{HP})_5\text{-}\beta\text{-CD}$ ) was isolated (isolation width,  $8.0 \text{ m/z}$ ) and fragmentation was performed via collision induced dissociation (CID) using an amplitude of  $0.65 \text{ V}$ . MS and MS/MS spectra were collected between 200 and  $2000 \text{ m/z}$ . Data were analyzed using the DataAnalysis software from Bruker.

HP- $\beta$ -CD eluted at about 7 min, whereas retained matrix compounds eluted between 8 and 16 min. Since the concentration of HP- $\beta$ -CD in the intestinal fluids was relatively high, samples were diluted 30–500 times in water prior to injection. Calibration curves were constructed by adding increasing concentrations of HP- $\beta$ -CD to blank human intestinal fluid, which was diluted 30 times in water (i.e. the minimum dilution factor required for the reliable quantification of HP- $\beta$ -CD in the samples). The slopes obtained for the calibration curves were not significantly different from the slopes obtained for measuring increasing HP- $\beta$ -CD concentrations in water, indicating matrix effects were virtually absent. The linear measuring range was demonstrated between 1 and  $250 \mu\text{g}/\text{mL}$  HP- $\beta$ -CD ( $r^2$  of 0.995) and the method was able to detect concentrations down to  $0.25 \mu\text{g}/\text{mL}$ . The method showed good intra- ( $n = 6$ ) and interday ( $n = 18$ , 6 consecutive injections on three different days) repeatability with RSDs (%) between 0.2 and 0.8% for retention times and between 4% and 8% for peak areas, respectively. Recovery values ranged from 95% to 100% for a spiked concentration of  $200 \mu\text{g}/\text{mL}$  HP- $\beta$ -CD.

## 2.5. Data presentation and statistical analysis

The Spearman's rank coefficient was used to quantify the correlation between the measured HP- $\beta$ -CD concentrations on the one hand

and the dissolved concentrations of itraconazole or the thermodynamic solubility on the other hand. To relate the precipitated fraction of itraconazole to the concentration of HP- $\beta$ -CD, only samples within 30 min of the  $t_{\text{max}}$  of itraconazole were included.

## 3. Results and discussion

In a previously performed small-scale clinical study, healthy volunteers were asked to ingest an oral solution of itraconazole (Sporano<sup>x</sup>) containing 40% HP- $\beta$ -CD (i) with or (ii) without a standardized volume of water (240 mL) after which gastrointestinal and blood samples were collected at predetermined time points. Although the intake of water resulted in substantially lower duodenal concentrations of itraconazole due to intraluminal dilution and increased extent of precipitation, plasma concentrations were almost unaffected [17]. It was hypothesized that these findings could be linked to differences in the extent of itraconazole entrapment in cyclodextrin complexes at different cyclodextrin concentrations [19]. Hence, a full understanding of these observations requires insight into the concentrations of HP- $\beta$ -CD in the intestinal fluids. Due to the limited volumes of intestinal fluids available, HP- $\beta$ -CD concentrations were only measured for a single volunteer (HV02); both test conditions (with and without water) were included in the analysis. Average concentrations ( $n = 5$ ) of itraconazole have already been reported in a previous publication [17]; in the present manuscript, however, we specifically refer to the concentrations of itraconazole for HV02.

### 3.1. Quantification of HP- $\beta$ -CD in human intestinal fluids

When a clinically relevant dose (20 mL) of Sporano<sup>x</sup> solution was administered to HV02 without water, the duodenal HP- $\beta$ -CD concentration-time profile as determined by LC-MS/MS closely followed the corresponding itraconazole profile, as illustrated in Fig. 1A. The maximum concentration of HP- $\beta$ -CD ( $81.3 \text{ mg}/\text{mL} = 8.1\%$ ) in the duodenum was measured 15 min after the intake of the 40% HP- $\beta$ -CD solution, corresponding to the  $t_{\text{max}}$  for itraconazole.

When the solution was coadministered with a standardized volume of water to the same volunteer, lower duodenal concentrations of HP- $\beta$ -CD were measured, as depicted in Fig. 1B. More specifically, the maximum concentration of HP- $\beta$ -CD amounted to  $20.5 \text{ mg}/\text{mL} (= 2.1\%)$  and was measured 90 min after the oral intake of the formulation. Unfortunately, due to the limited sample volume, the concentration of HP- $\beta$ -CD could not be determined when the maximum concentration of itraconazole ( $t = 60 \text{ min}$ ) was attained for this test condition (Fig. 1B, dotted line).

Overall, when comparing the measured concentrations of

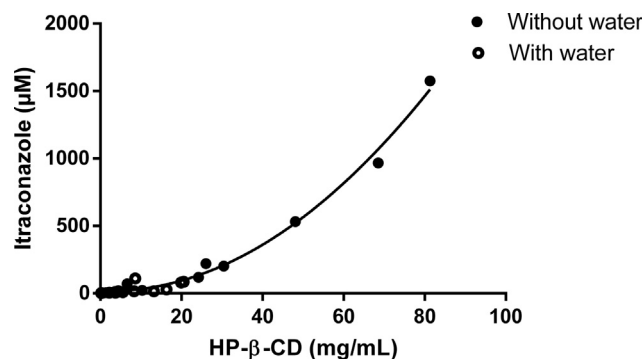


Fig. 2. Correlation between measured concentrations of itraconazole and HP- $\beta$ -CD in duodenal fluids following the oral administration of Sporanox<sup>®</sup> with (open circles) and without (closed circles) 240 mL of water to HV02. Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).

itraconazole and HP- $\beta$ -CD in the duodenal fluids for both test conditions, an excellent correlation was observed, which was characterized by a Spearman's rank coefficient of 0.96, as illustrated in Fig. 2. Although dissolved intestinal itraconazole concentrations can be influenced by physiological factors such as pH and bile salt concentrations, the strong correlation in Fig. 2 indicates that the HP- $\beta$ -CD concentration is the most factor component determining the itraconazole concentration in the intraluminal environment upon the oral administration of Sporanox<sup>®</sup> solution.

Interestingly, when comparing the ratio (“without water” over “with water”) of the area under the duodenal concentration-time curve ( $AUC_{0-3h}$ ) for HP- $\beta$ -CD and dissolved concentrations of itraconazole, this ratio was remarkably higher for itraconazole (6.4-fold difference) than for HP- $\beta$ -CD (2.3-fold difference). The high ratio for the dissolved concentrations of itraconazole can be explained by the reported increase in precipitation of itraconazole upon water intake [17]. In contrast, for the total content of itraconazole (dissolved and precipitated), a ratio (2.4-fold difference) similar to the one calculated for HP- $\beta$ -CD was observed indicating that HP- $\beta$ -CD and total itraconazole were diluted to the same extent.

Furthermore, calculated intestinal HP- $\beta$ -CD concentrations, assuming a residual gastric volume of 50 mL and a 1:1 dilution upon gastrointestinal transfer, approach measured peak concentrations of HP- $\beta$ -CD for both test conditions: in the case of concomitant water intake, a concentration of 1.3% (vs. 2.1% as experimental concentration) was calculated while for the administration of the formulation without water, an intestinal HP- $\beta$ -CD peak concentration of 5.7% (vs. 8.1% as experimental concentration) was predicted.

### 3.2. Itraconazole solubility in intestinal fluids containing HP- $\beta$ -CD

In addition to the concentration of itraconazole, the thermodynamic solubility of itraconazole (24 h, 37 °C) was also determined for each individual duodenal aspirate of HV02 [17]. As thoroughly described in literature, *in vitro* experiments in water and several aqueous buffer systems (including a pH 4 and 7 phosphate-citrate buffer) clearly demonstrate a positive deviation from linearity for the relationship between itraconazole solubility and concentrations of HP- $\beta$ -CD, which is known as an  $A_p$ -type profile [19,22]. Instead of a 1:1 association, this type of profile suggests that one itraconazole molecule interacts with several cyclodextrin units meaning that higher order complexes are formed at higher concentrations of HP- $\beta$ -CD. Additionally, Stappaerts et al. observed similar  $A_p$ -type profiles when assessing the impact of HP- $\beta$ -CD on the solubility of itraconazole in biorelevant media including fasted and fed state simulated intestinal media (FaSSIF/FaSSIF) [20]. As illustrated in Fig. 3, our data suggest that such a relationship also exists in fasted state human intestinal fluids (Spearman's rank coefficient of

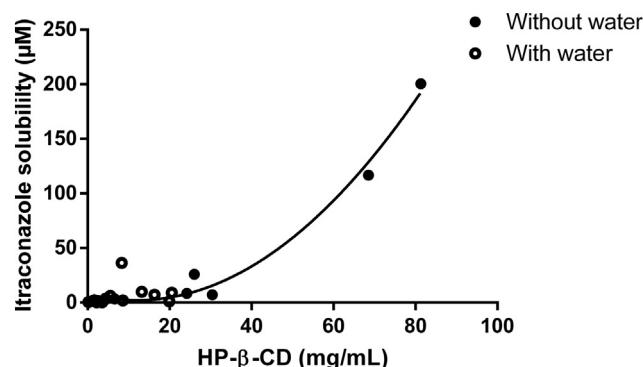


Fig. 3. Itraconazole solubility in human intestinal fluids as a function of concentration of HP- $\beta$ -CD. Circles represent an individual duodenal aspirate following the administration of Sporanox<sup>®</sup> solution to HV02 without (closed) and with (open) water, respectively. Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).

0.78).

### 3.3. Interpreting intraluminal drug behavior

The measured HP- $\beta$ -CD concentrations in the intestinal samples might help to understand the gastrointestinal observations upon administration of the cyclodextrin-based solution of itraconazole with or without a glass of water. Upon gastrointestinal transfer of the weakly basic drug from the acidic environment of the stomach into the more neutral environment of the small intestine, supersaturated concentrations of itraconazole were observed in the duodenum, irrespective of water intake [17]. Although supersaturation of itraconazole might be stabilized by the presence of cyclodextrins [21,23], it is unstable from a thermodynamic point of view and therefore the driving force for precipitation. The extent of intestinal precipitation was significantly increased when the formulation was coadministered with water compared to the intake as such. For HV02, for instance, the percentage of intestinal precipitation, based on the difference of the dissolved and total concentration-time profile ( $AUC_{0-3h}$ ) of itraconazole, amounted to 66.7% with water intake vs. 13.2% without water intake. This discrepancy is most likely related to an increased intraluminal dilution of the solubilizing cyclodextrins that are present in the formulation. Indeed, considering the samples around the  $t_{max}$  of itraconazole ( $\pm 30$  min), the extent of precipitation could be linked reasonably well with the measured concentration of HP- $\beta$ -CD (Fig. 4). This observation clearly implies that, as a result of the non-linear phase-solubility profile (Fig. 3), higher order complexes are formed at higher concentrations of

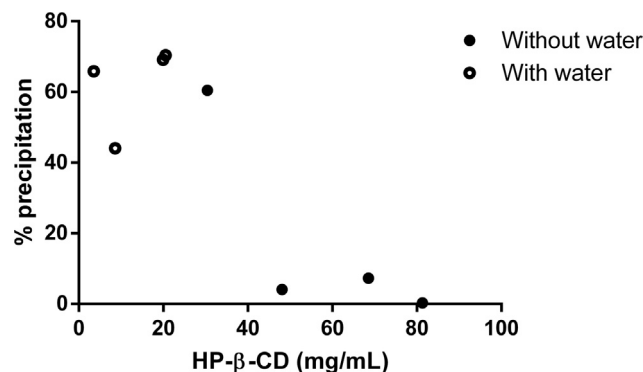


Fig. 4. Percentage precipitation of itraconazole as a function of the measured concentration of HP- $\beta$ -CD in aspirated duodenal fluids. Circles represent an individual duodenal aspirate following the administration of the formulation to HV02 without (closed) and with (open) water, respectively. Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).



HP- $\beta$ -CD explaining the lower precipitated fraction of itraconazole at higher concentrations of HP- $\beta$ -CD. Upon strong intraluminal dilution due to water intake, weaker complexes are formed and itraconazole precipitation is more pronounced. To quantify the difference in affinity between itraconazole and HP- $\beta$ -CD at varying concentrations of HP- $\beta$ -CD, actual stability constants would be of great interest; however, it is not evident to calculate a stability constant in complex media such as intestinal fluids since the equilibrium between itraconazole and cyclodextrins will be affected by interactions between itraconazole and bile salt/phospholipid micelles, and between bile salts and cyclodextrins. As the composition of intestinal fluids is continuously changing over time and the intestinal samples were aspirated time-dependently, these interactions will differ in each intestinal aspirate. Moreover, pH highly affects ionization behavior of itraconazole and as a result the affinity for complexation with HP- $\beta$ -CD. Since pH fluctuated (range 3.5–7.7) among the different intestinal aspirates, this implies that the interaction between itraconazole and HP- $\beta$ -CD differs in each intestinal aspirate, again complicating the calculation of a meaningful stability constant.

Despite the fact that the data strongly support the importance of cyclodextrins in stabilizing itraconazole solutions in the duodenum, it should be emphasized that other factors (e.g. bile salts) may affect the dissolved fraction as well [20].

In contrast to the remarkable impact of water intake on duodenal itraconazole concentrations, the combined systemic exposure of itraconazole and its active metabolite hydroxy-itraconazole was almost unaffected, as reported previously [17]. In HV02, systemic concentrations were even decreased when the formulation was administered without water (Fig. 5), despite a strong increase in duodenal concentrations (6.4-fold). Presumably, the stronger interaction at higher HP- $\beta$ -CD concentrations reduces the free fraction of the drug that is available to cross the intestinal epithelium. Indeed, higher HP- $\beta$ -CD concentrations were measured when Sporanox® was administered without water invigorating the hypothesis that the extent of entrapment of itraconazole in the concentration-dependent cyclodextrin complexes determines the intestinal absorption. It should be noted that HP- $\beta$ -CD is not absorbed from the gastrointestinal tract due to its size and hydrophilic nature [24].

To directly explore the influence of HP- $\beta$ -CD on the intestinal permeation of itraconazole, aliquots (500  $\mu$ L) of freshly aspirated duodenal fluids were, as previously reported [17], immediately applied to the donor side of a Caco-2 cell system. Since the concentrations of HP- $\beta$ -CD were unknown at the time of the initial study, permeated itraconazole data were only suggestive for a higher extent of entrapment at higher HP- $\beta$ -CD concentrations. Based on the measured concentrations of HP- $\beta$ -CD in the intestinal samples, the hypothesis that higher HP- $\beta$ -CD concentrations reduce the free drug fraction appears to be justified. As

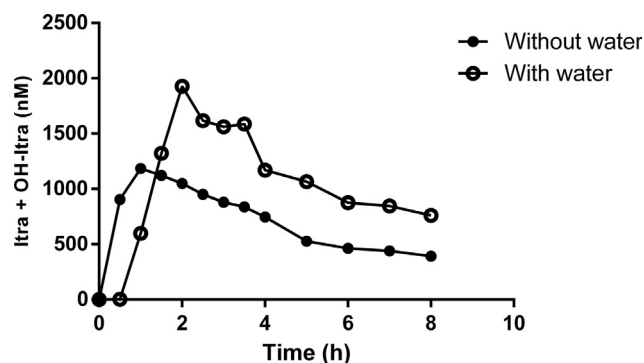


Fig. 5. Summed plasma concentration-time profile of itraconazole and hydroxy-itraconazole for a single volunteer (HV02) following the oral administration of Sporanox® solution with (open circles) or without (closed circles) a glass of water. Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).

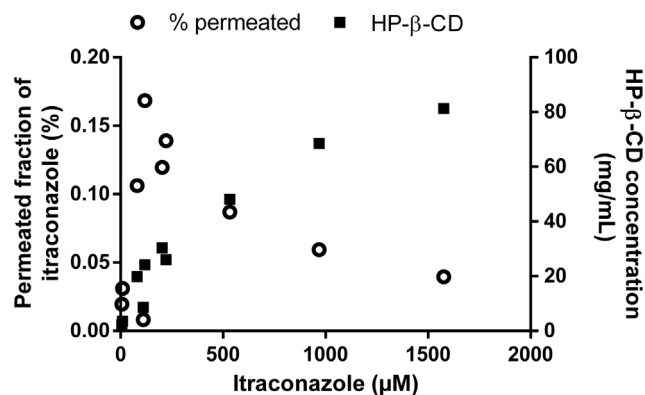


Fig. 6. Relationship between percentages of itraconazole permeated (relative to the amount present in the donor compartment) across Caco-2 cells after 30 min and the dissolved concentration of itraconazole in the aspirated duodenal fluids at the donor side. Open circles and closed squares represent for each individual aspirate from HV02 the percentage of itraconazole permeated across a Caco-2 cell monolayer (left y-axis) and the measured concentration of HP- $\beta$ -CD (right y-axis), respectively. Concentrations of itraconazole are adopted from Berben et al. [17] (with permission).

illustrated in Fig. 6, high HP- $\beta$ -CD concentrations were measured in samples where a lower percentage of itraconazole (relative to the amount present in the donor compartment) reached the basolateral medium indicating that an increase in donor itraconazole concentration did not result in a proportionally increased transport of itraconazole. Conversely, lower HP- $\beta$ -CD concentrations were accompanied with a higher permeated fraction of itraconazole. As such, these data affirm the impact of cyclodextrins on the intestinal permeation of itraconazole by the formation of concentration-dependent inclusion complexes that impede the intestinal permeation of the lipophilic drug.

Overall, the current data established that quantification of HP- $\beta$ -CD concentrations in the intestinal samples is essential to interpret the intraluminal behavior of itraconazole upon oral administration of a highly concentrated HP- $\beta$ -CD-based solution with a different volume of water (0 mL vs. 240 mL). Moreover, the data obtained substantiate the hypothesis that cyclodextrins are able to enhance intraluminal concentrations of itraconazole but may simultaneously reduce the free fraction of the drug available for permeation.

#### 4. Conclusion

Since pharmaceutical excipients may fundamentally affect gastrointestinal drug behavior, in-depth gastrointestinal formulation evaluation benefits from their assessment. In this respect, concentrations of the solubilizing agent HP- $\beta$ -CD were quantified, for the first time, in human duodenal fluids upon the oral administration of a highly concentrated HP- $\beta$ -CD solution. Linking the intestinal concentrations of HP- $\beta$ -CD to itraconazole concentrations helps to understand intraluminal formulation behavior as illustrated by the fact that intraluminal itraconazole concentrations are not necessarily freely available for permeation in the presence of cyclodextrins by the formation of inclusion complexes. Since knowledge about physiologically relevant HP- $\beta$ -CD concentrations in the small intestine is limited, the measured concentrations of HP- $\beta$ -CD might be useful as reference data to support the development of predictive *in vitro* and *in silico* tools.

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